

Int'l Appl. No. : PCT/JP2004/015174
Int'l filing date : October 14, 2004

AMENDMENTS TO THE SPECIFICATION

Please amend the title as shown:

PROCESS-METHOD FOR PRODUCING SCYLLO-INOSITOL

On page 1 of the Specification, after the Title of the Invention and before the Technical Field starting on line 1, please insert the following section:

Related Applications

This application is the U.S. National Phase under 35 U.S.C. § 371 of International Application PCT/JP2004/015174, filed October 14, 2004, which claims priority to JP 2003-353490, filed October 14, 2003; JP 2003-353491, filed October 14, 2003; JP 2004-018128, filed January 27, 2004; and JP 2004-194088, filed June 30, 2004.

Please correct the following numbered paragraphs as shown:

[0237]

Next, to confirm the scyllo-inositol dehydrogenase activity, the microbial strains isolated as colonies were transferred to 100 ml of LB medium (1% bacto-tryptone, 0.5% yeast extract, 1% NaCl, pH 7.0) containing 50 µg/ml ampicillin, and they were cultured at 36°C for 7 hours. 0.3 ml of 200 mM thiogalactopyranoside solution was added to the culture solution, and the cells were further cultured at 36°C for 3 hours. After completion of the culture, the cells were collected by centrifugation and washed with physiological saline once. Then, the washed cells were suspended in 3 ml of 0.6% Triton X-100 solution, and the cells were disrupted by ultrasonic wave at 4°C. The solution was centrifuged, and 2.8 ml of the supernatant (enzyme solution) was taken out. 1.2 g of ammonium sulfate was added to the supernatant to salt out proteins at 4°C. The salted-out proteins were collected by centrifugation (15,000 rpm, 20 min), and the supernatant was removed. The precipitates were dissolved in 2.5 ml of 20 mM Tris buffer (pH 7.0), and the solution was centrifuged (15,000 rpm, 20 min) again. The supernatant was applied onto ~~Shephadex~~Sephadex G-25 column (Pharmacia K.K.: 14 ml) equilibrated with 20 mM Tris buffer (pH 7.0). Elution was performed with 20 mM Tris buffer (pH 7.0), and the

eluate was desalted. The procedures yielded 3.5 ml of a crude enzyme solution of ydgJ gene product.

[0252]

Next, to confirm the scyllo-inositol dehydrogenase activity, the microbial strains isolated as colonies were transferred to 100 ml of an LB medium (1% bacto-tryptone, 0.5% yeast extract, 1% NaCl, pH 7.0) containing 50 µg/ml ampicillin, and they were cultured at 36°C for 7 hours. 0.3 ml of 200 mM thiogalactopyranoside solution was added to the culture solution, and the cells were further cultured at 36°C for 3 hours. After completion of the culture, the cells were collected by centrifugation and washed with physiological saline once. Then, the washed cells were suspended in 3 ml of 0.6% Triton X-100 solution, and the cells were disrupted by ultrasonic wave at 4°C. The solution was centrifuged, and 2.8 ml of the supernatant (enzyme solution) was taken out. 1.2 g of ammonium sulfate was added to the supernatant to salt out proteins at 4°C. The salted-out proteins were collected by centrifugation, and the supernatant was removed. The precipitates were dissolved in 2.5 ml of 20 mM Tris buffer (pH 7.0), and the solution was centrifuged again. The supernatant was applied onto a ~~Shephadex~~Sephadex G-25 column (14 ml) equilibrated with 20 mM Tris buffer (pH 7.0). Elution was performed with 20 mM Tris buffer solution (pH 7.0), and the eluate was desalted. The procedures yielded 3.5 ml of a crude enzyme solution of ydgJ gene product.

[0279]

Next, to confirm the scyllo-inositol 2-dehydrogenase activity, the cells isolated as colonies were transferred into 30 bottles of 100 ml of an LB medium (1% bacto-tryptone, 0.5% yeast extract, 1% NaCl, pH 7.0) containing 50 µg/ml ampicillin, and they were cultured at 36°C for 7 hours. 0.3 ml of 200 mM thiogalactopyranoside solution was added to each 100ml of the culture solution, and the cells were further cultured at 36°C for 3 hours. After completion of the culture, the cells were collected by centrifugation and washed with physiological saline once. Then, the washed cells were suspended in 3 ml of 0.6% Triton X-100 solution, and the cells were disrupted by ultrasonic wave at 4°C. The solution was centrifuged, and 84 ml of the supernatant

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(enzyme solution) was taken out. 36 g of ammonium sulfate was added to the supernatant to salt out proteins at 4°C. The resultant proteins were collected by centrifugation, and the supernatant was removed. The precipitates were dissolved in 75 ml of 20 mM Tris buffer (pH 7.0), and the solution was centrifuged again. The supernatant was applied onto ~~Sephadex~~ G-25 column (Pharmacia K.K.)(400ml) equilibrated with 20 mM Tris buffer (pH 7.0), and elution was performed with 20 mM Tris buffer (pH 7.0). Elution was performed with 20 mM Tris buffer (pH 7.0), and the eluate was desalting. The procedures yielded 105 ml of a crude enzyme solution of BG10669 gene product.

On page 100 before Claim 1, please amend as follows:

WHAT IS CLAIMED IS: CLAIMS